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**Phenolic antioxidants inhibit the triplet-induced transformation of
anilines and sulfonamide antibiotics in aqueous solution**

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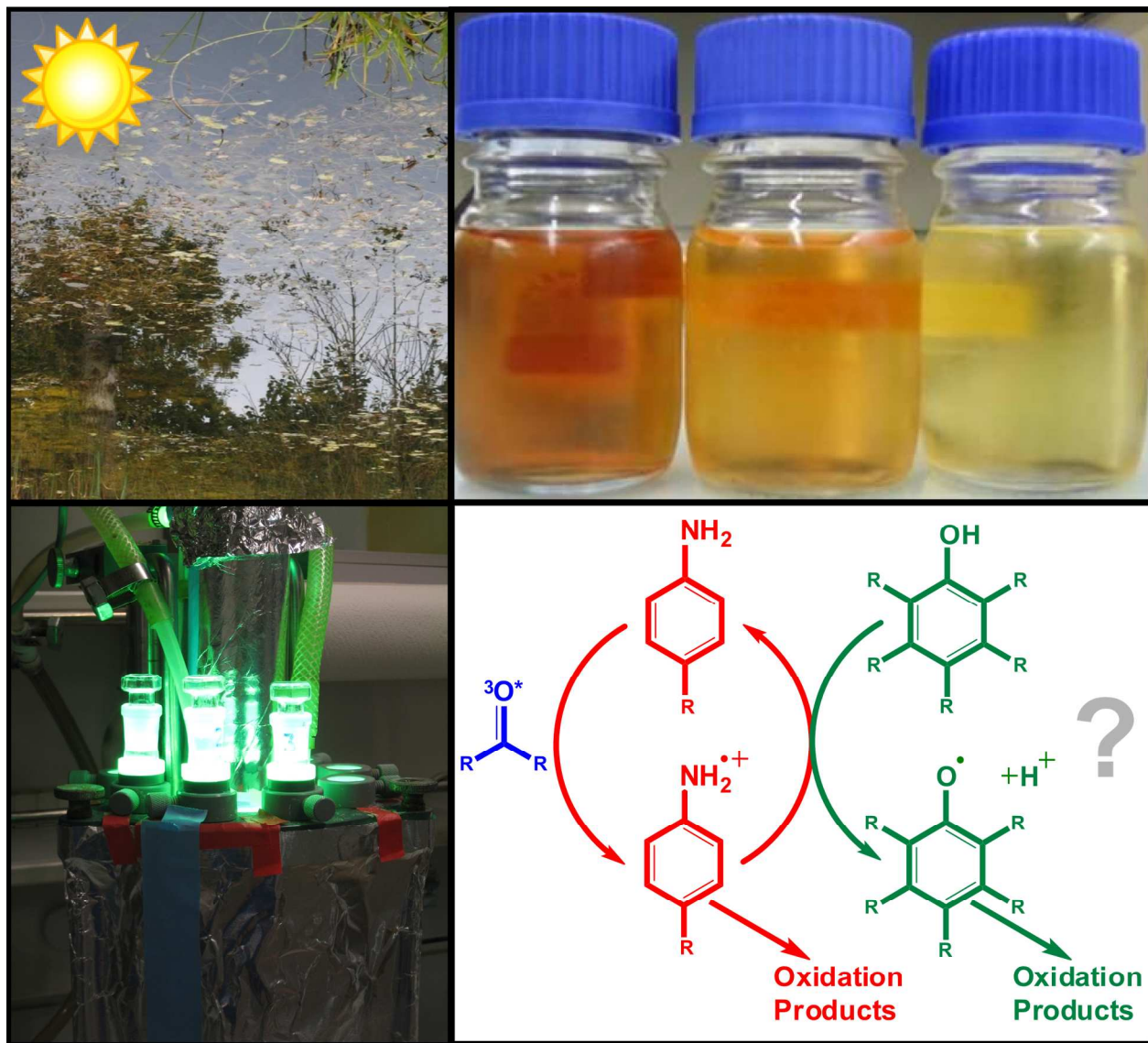
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Abstract

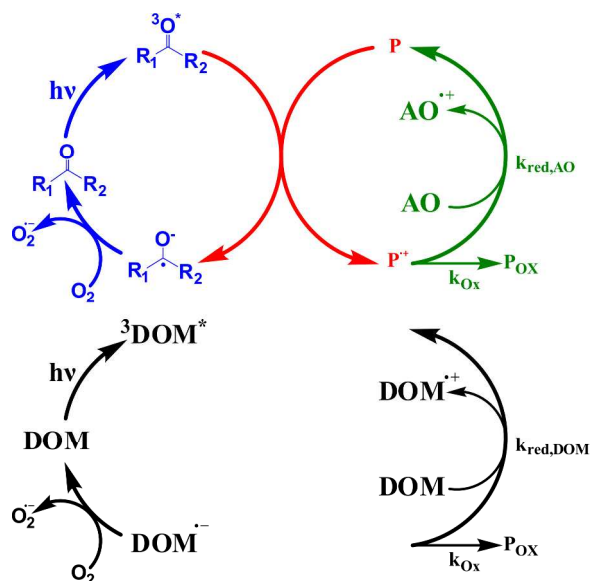
Recent studies have shown that dissolved organic matter (DOM) may inhibit the excited triplet-induced oxidation of several aromatic water contaminants, in particular those containing an aniline functionality. Such an inhibition was ascribed to antioxidant moieties of DOM. The present study was conducted with the aim of verifying whether well-defined antioxidants could act as inhibitors in analogy to DOM. Various substituted phenols exhibiting antioxidant character were able, at micromolar concentration, to slow down the photo-induced depletion of several anilines and sulfonamides in aerated aqueous solution containing 2-acetonaphthone as the photosensitizer. A concomitant accelerated degradation of the phenols in the presence of such contaminants was observed. This reinforces the hypothesis of reduction of oxidation intermediates of the contaminants by the phenols. Phenol (unsubstituted) was found to be a useful inhibitor even in the case of DOM-photosensitized transformations. Phenolic antioxidants are proposed as diagnostic tools to investigate the aquatic photochemistry of aromatic amines.

Introduction

Oxidation induced by reaction with photo-generated excited triplet states is important for the fate of contaminants in sunlit surface waters.¹ The source of these excited triplet states is constituted by the chromophoric components of dissolved organic matter (DOM), which are generally the main absorber of sunlight in surface waters.² Moreover, DOM is involved as a photosensitizer in other reactions leading to an enhanced transformation of contaminants.³ However, DOM may also play an opposite role and slow down the sunlight-induced transformation of contaminants by various mechanisms. Besides light screening and scavenging of photooxidants (e.g. hydroxyl radical⁴⁻⁶ and carbonate radical^{7, 8}), DOM has been shown to inhibit the excited triplet-induced transformation of certain aromatic compounds. In two preceding studies^{9, 10} we characterized these inhibition reactions by employing two aromatic ketones as excited triplet state precursors and using various types of DOM as inhibitors. Compounds containing amine moieties, in particular the broad class of the anilines, including 4-aminophenyl-sulfonamides that are used as antibiotics (simply referred to as sulfonamides in the following), were significantly affected by inhibition. The observed inhibition generally increased with DOM concentration and could be explained in terms of a one- or two-channel reaction model.¹⁰ DOM of terrestrial origin with high aromaticity was shown to be a better inhibitor than DOM of aquatic origin with low aromaticity. To interpret the findings we proposed that an oxidation intermediate (possibly but not necessarily the radical resulting from one-electron oxidation of the target compound) was reduced by some antioxidant moieties present in the DOM. The occurrence of such moieties is likely because DOM, in particular its humic fraction, possesses a relevant electron donating capacity (EDC)^{11, 12} and is even capable to reduce mild oxidants such as ferric citrate and hexacyanoferrate.¹³ The chemical complexity of DOM impairs

the direct identification of the antioxidant moieties thought to be responsible for the observed inhibition effect. However, probable candidates may be found in the class of phenolic moieties, which are abundant in DOM.^{14, 15} Phenols, especially those bearing electron-rich substituents, are known to exhibit antioxidant activity.¹⁶ Phenolic antioxidants (AOs) are either present in nature to prevent oxidative damage to living cells, as in the case of α -tocopherol (vitamin E) and various phenolic compounds produced in plants, or added on purpose to inhibit the degradation of numerous goods such as foods, pharmaceuticals and personal care products, and plastic materials.¹⁷

The utilization of model AOs offers new options to explore the inhibition of triplet-induced oxidation, as illustrated in Scheme 1. The left-hand part of the scheme shows the two alternatives we have used to date to induce oxidations by excited triplet states. Chromophores of either a model aromatic ketone or DOM, both acting as photosensitizers, are promoted by absorption of light to their excited triplet states, which oxidize a contaminant (P) to an intermediate contaminant radical cation ($P^{\bullet+}$, representing here for simplicity a suite of possible oxidation intermediates). The model photosensitizer or DOM are recycled via formation of a radical anion and subsequent reaction with molecular oxygen, which takes up the exceeding electron thus forming the superoxide radical anion ($O_2^{\bullet-}$). The right-hand part of the scheme considers two alternatives for inhibition of oxidation, consisting in the reduction of the oxidation intermediate $P^{\bullet+}$, either by reaction with a model AO or with DOM, yielding back the parent contaminant P. The reduction reaction competes with the formation of oxidation products (P_{Ox}) which cannot react back to give P.



Scheme 1. Conceptual model for the triplet-induced oxidation of a contaminant (P) and subsequent inhibition: Alternative systems with model and natural photosensitizers, and with model and natural antioxidants/inhibitors.

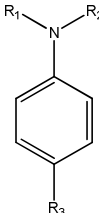
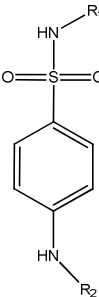
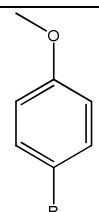
There are four different ways to combine the various photosensitizer and inhibitor reaction elements: (1) A model aromatic ketone is used as the photosensitizer in combination with a model AO (upper part of Scheme 1); (2) DOM is used as the photosensitizer together with a model AO as the inhibitor (black arrows in the lower left part combined with green arrows); (3) A model aromatic ketone is used in combination with DOM as the inhibitor (blue arrows combined with black arrows in the lower right part); (4). DOM is both the photosensitizer and the inhibitor in the oxidation of P (lower part of the scheme, black arrows). This last combination mode represents the situation occurring in a DOM solution or a natural water, but is not suitable for mechanistic investigations, so it is not further considered in this paper. In our previous studies^{9, 10} the combination mode (3) was employed. The application of the combination modes (1) and (2) are presented for the first time in this paper.

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3 111 The main objective of the present study was to demonstrate that phenolic compounds (as
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5 112 model AOs) are able to inhibit the triplet-induced oxidative transformation of certain
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8 113 contaminants as observed for DOM, thus providing a proof of concept for the proposed
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10 114 mechanistic interpretation. This was achieved by conducting steady-state irradiation experiments
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12 115 of solutions containing a given target contaminant and appropriate combinations of
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14 116 photosensitizers and model AOs. The depletion kinetics of both target contaminant and AO was
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16 117 determined for each single experiment.
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19 118 For a successful implementation, particular attention had to be paid to the selection of the
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21 119 target compounds, the model AOs and the model photosensitizers. As target compounds, several
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23 120 anilines and sulfonamides were chosen, because these classes of compounds have proven to be
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25 121 significantly affected by inhibition of oxidation.^{9, 10} Moreover, anilines represent basic functional
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27 122 units of many water contaminants,¹⁸ and sulfonamide antibiotics are frequently detected aquatic
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29 123 contaminants,¹⁹ the photochemical transformation of which is of great interest.²⁰⁻²³ Little is
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31 124 known about the inhibiting effect of phenols on photoinduced reactions.²⁴⁻²⁶ In selecting the
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33 125 model phenolic AOs, the general ability of phenols to undergo direct^{27, 28} and indirect²⁹⁻³⁴
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35 126 phototransformation in water was considered. As main phenolic antioxidants to be used in this
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37 127 study phenol and 4-methylphenol were preferred, due to their relative stability against triplet-
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39 128 induced oxidation³² and the consequent reduction of the risk of interference by side-reactions, but
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41 129 we also extended our selection to include several hydroxybenzoic acids, because they probably
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43 130 better match the chemical structure and reactivity of the phenolic units present in DOM. 2-
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45 131 Acetonaphthone (2AN) was chosen as the model photosensitizer due to the low reactivity of its
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47 132 excited triplet state toward phenols.³⁵
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134 **Table 1. Substances tested in this study**

(a) anilines			(d) phenols (including hydroxybenzoic acids)		
	aniline	ANI	phenol		
	4-chloroaniline	4CA	4-methylphenol		4MP
	<i>N</i> -methylaniline	NMA	catechol		
	<i>N,N</i> -dimethylaniline	DMA	resorcinol		
(b) sulfonamides					
	sulfadiazine	SD	L-tyrosine		TYR
	sulfathiazole	STZ	p-coumaric acid		CMA
	sulfamethoxazole	SMX	3-hydroxybenzoic acid		3HBA
	sulfachloropyridazine	SCPD	4-hydroxybenzoic acid		4HBA
	acetylsulfadiazine	ASD	3,4-dihydroxybenzoic acid		3,4DHBA
	acetylsulfathiazole	ASTZ	2,4-dihydroxybenzoic acid		2,4DHBA
(c) anisoles					
	anisole		5-hydroxy-2-methylbenzoic acid		5H2MBA
	4-methylanisole	4MA	phenolic controls	2,4,6-trimethylphenol	TMP
				3,4-dimethoxyphenol	DMOP
				trolox	

137 Experimental Section

138 **Materials and chemicals.** The organic substances used in this study and their abbreviations
139 are shown in Table 1 and more comprehensively in the Supporting Information (SI, Figure S1).
140 Anilines (a) and sulfonamides (b) served as target compounds (TCs) for the photosensitized
141 oxidation experiments. Phenols (d), including hydroxybenzoic acids, were mostly employed as
142 antioxidants (AOs). However, TMP, DMOP and trolox did not serve as AOs but as control TCs.
143 These electron-rich compounds were expected not to undergo inhibition of oxidation⁹ and were
144 therefore used as indicators of possible side-reactions. Two anisoles (c) were chosen as non-AO
145 controls. For a complete list of all chemicals used and preparation of solutions see the SI, Text S1.
146 Standard DOMs: Nordic Aquatic Fulvic Acid (NAFA), Pony Lake Fulvic Acid (PLFA), and
147 Suwannee River Fulvic Acid (SRFA) were obtained from the International Humic Substances
148 Society. Freshwater samples (all from Switzerland) were taken from: 1) River Murg, Switzerland
149 upstream (Latitude: 47°34'4.66"N; Longitude: 8°53'41.34"E; [DOM]=1.9 mg C L⁻¹; pH 8.1) and
150 downstream (47°34'43.42"N; 8°53'9.04"E; [DOM]=2.3 mg C L⁻¹, pH 8.1) of Frauenfeld's
151 municipal waste water treatment plant (WWTP); 2) River Thur (47°35'27.26"N; 8°46'19.81"E;
152 [DOM]=2.3 mg C L⁻¹; pH 8.2); 3) Lake Greifensee effluent (47°22'44.20"N; 8°38'47.82"E;
153 [DOM]=3.8 mg C L⁻¹; pH 8.0). Samples were immediately filtered (0.45 µm, cellulose nitrate,
154 Sartorius AG, Goettingen, Germany) and stored in glass bottles at 4°C. Experiments were
155 conducted within four weeks after sample collection.

156 **Irradiation experiments.** Irradiation conditions are described in detail elsewhere.¹⁰ A central
157 requirement for the present study was to avoid quenching of the excited triplet state of 2AN
158 (³2AN*) by the added model AO, to ensure that triplet quenching was not the cause of any
159 decreased transformation rates of TCs. For any AO a maximum second-order quenching rate

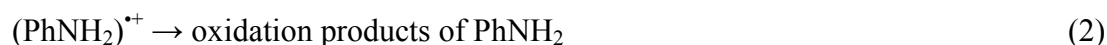
constant of $3.1 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ can be assumed³⁵, which translates into a first-order quenching constant of $3.1 \times 10^4 \text{ s}^{-1}$ for the generally employed AO concentration of 10 μM . Comparing this constant with the deactivation constant of $^3\text{2AN}^*$ in aerated aqueous solution ($6.44 \times 10^5 \text{ s}^{-1}$)³⁵, one obtains a maximum quenching contribution of 4.6 % due to the presence of AO. With phenol and 4MP the quenching contribution for 10 μM is even much below this value (0.05 % and 0.13 %, respectively), and is still small even at an AO concentration of 500 μM (2.5 % and 6.1 %, respectively). We therefore conclude that quenching by AO in any of the experiments performed in this study was lower than the experimental uncertainty (typically ≈ 15 %) and thus negligible.

Irradiation experiments were performed in quartz glass tubes at a temperature of 25.0 ± 0.5 °C. The irradiated solutions (20 mL) were buffered at pH 8.0 (5 mM phosphate) except for the case of the natural waters (which had a pH near 8). The solutions contained various combinations of a TC (5 μM), a sensitizer (either 2AN at 10 – 50 μM , or standard DOMs, $[\text{DOM}] = 2.5 - 5 \text{ mg C L}^{-1}$, or the DOM of the natural waters), an AO (10 μM for the majority of irradiations and 1 – 750 μM for the study of the concentration dependence) and a control compound. For a full overview of all combinations see Text S3 and Table S3 in the SI. Samples of 400 μL were withdrawn from each tube at six equidistant time intervals during irradiation.

Analytical methods. High-performance liquid chromatography (HPLC) using UV-vis absorbance and fluorescence detection was employed to quantify the concentration of organic compounds over the course of the irradiations. Details on the HPLC equipment and methods, and on pH and spectrophotometric measurements are given in the SI, Text S2, Table S1 and S2.

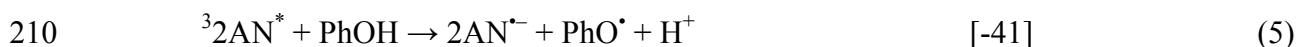
Results and Discussion

Phototransformation of target compounds (TCs) induced by excited triplet 2-acetonaphthone (2AN): Inhibition by phenolic antioxidants (AOs). The depletion kinetics of each of the studied TCs (anilines and sulfonamides) and the AOs was followed in sets of steady-state irradiation experiments as illustrated in Figure 1. The behavior shown for aniline as a TC and phenol as an AO is typical for all studied TC/AO pairs (see SI). Aniline (Figure 1a) is efficiently depleted in the presence of 2AN, and the presence of phenol (10 μM) strongly inhibits the reaction. Moreover there is negligible aniline depletion in the absence of 2AN (with or without phenol present) and no inhibitory action of anisole (used as a negative control for the antioxidant activity). Phenol (Figure 1b) is appreciably depleted only in the presence of both 2AN and aniline, while it is not in the absence of one of these components. Summarizing, phenol inhibits the transformation of aniline, which, at the same time, catalyzes the transformation of phenol. Since the quenching of $^32\text{AN}^*$ by phenol is negligible (see Experimental Section), only mechanisms involving interaction of phenol with transients formed from aniline transformation may explain the observed behavior. We postulate the following reaction sequence (aniline = PhNH_2 ; phenol = PhOH ; $\Delta G^\circ/(\text{kJ mol}^{-1})$ in square brackets).

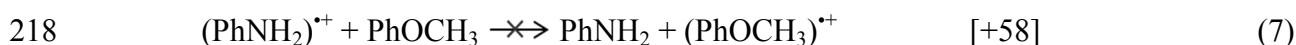


Note that reactions 1 and 3 are both exergonic at pH 8.0, since the standard one-electron reduction potential (all the following values in water, vs. NHE) of $^32\text{AN}^*$ (1.34 V³⁶) is higher than that of $(\text{PhNH}_2)^{+\cdot}$ (1.02 V³⁷), which in turn is higher than that of $(\text{PhO}^\cdot/\text{H}^+)$ at pH = 8.0 (0.91

V, calculated according to Li and Hoffman³⁸). In accordance with this, the reaction of $^3\text{2AN}^*$ with phenol (eq. 5) is even more exergonic (at pH 8.0) than the reaction of $^3\text{2AN}^*$ with aniline, but its kinetics is slow, since the rate-determining step is an endergonic electron transfer reaction (eq. 6).³⁵



We would like to point out that eq. 3 is written in a simplified form, since the aniline radical cation $(\text{PhNH}_2)^{\bullet+}$ can deprotonate yielding the corresponding conjugate base, $(\text{PhNH})^{\bullet}$. However, taking this acid base equilibrium into account ($\text{pK}_a = 7.05$)³⁹, the reaction is still exergonic and the same conclusions hold. The missing inhibitory effect in the presence of anisole can also be explained based on thermodynamic arguments. For anisole (PhOCH_3), eq. 3 is substituted by eq. 7:



This reaction is highly endergonic and hence extremely slow, owing to the high reduction potential of $(\text{PhOCH}_3)^{\bullet+}$ (1.62 V⁴⁰). Consequently, $(\text{PhNH}_2)^{\bullet+}$ is not reduced by anisole.

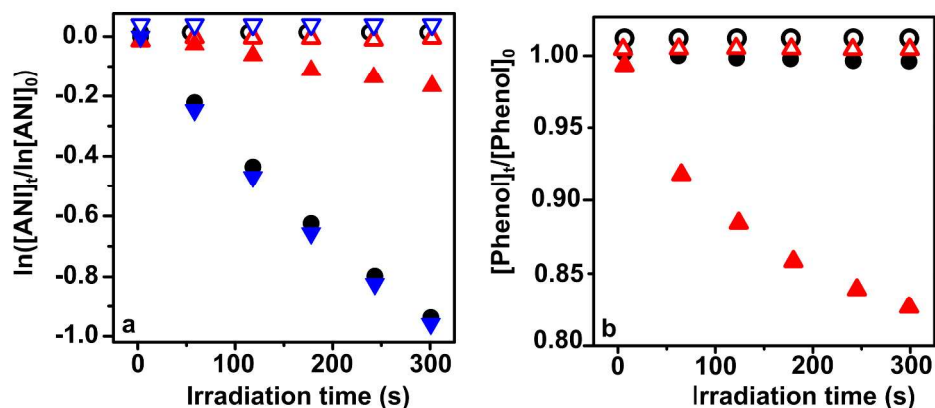


Figure 1. Depletion kinetics of (a) aniline (ANI) (5 μ M) and (b) phenol (10 μ M) in irradiated aqueous solutions (pH 8.0), in the presence (closed symbols) and absence (open symbols) of the photosensitizer 2-acetonaphthone (25 μ M). Solution composition: (●, ○) either aniline or phenol, (▼, ▽) aniline and anisole (10 μ M), (▲, △) aniline and phenol.

The representative example of the inhibited aniline transformation in the presence of phenol serves as a proof of concept and indicates that such a system can mimic the inhibition of triplet-induced oxidation observed in the presence of DOM.^{9, 10} Since the mentioned inhibition by DOM was observed with various target compounds, and since DOM is expected to include a great variety of phenolic AOs, investigations were extended to consider a series of TCs and a series of phenolic AOs, comprising some natural compounds. Firstly, for each TC, kinetic experiments as shown in Figure 1 were performed, using either phenol or 4-methylphenol as the AO (see SI, Figures S2 – S10). In a second series of experiments (see SI, Figures S11 – S21) the TC (aniline) was fixed, while different AOs were tested. The results of these experiments are shown in Figure 2. The pseudo-first-order rate constants for the depletion of each TC in the presence, $k_{Sens,AO}$, and

absence, k_{Sens} , of a given AO (the subscript *Sens* is used to denote the used photosensitizer) were determined and the inhibition efficiency (IE) was calculated according to eq. 8.

$$IE = 1 - k_{Sens,AO} / k_{Sens} \quad (8)$$

Rate constants for the depletion of each AO were also determined.

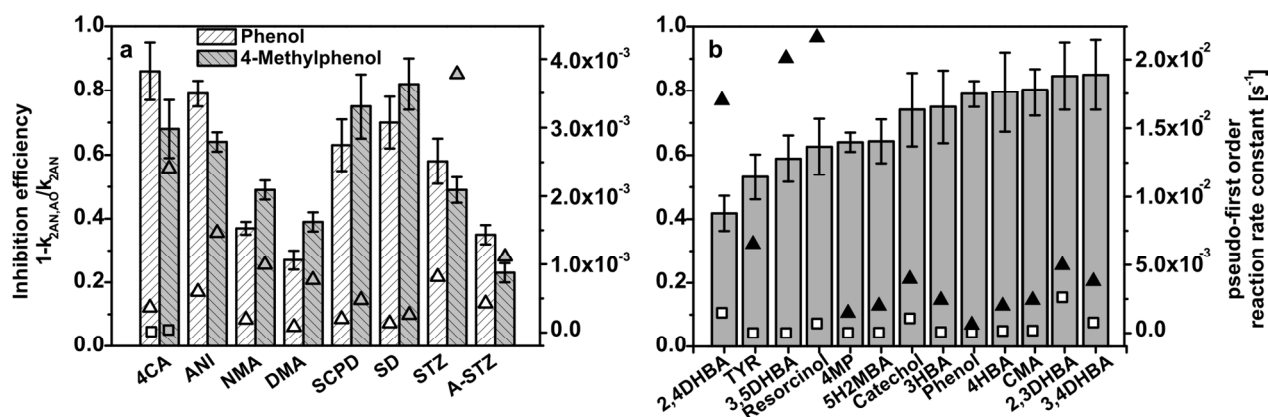


Figure 2. Inhibition efficiency (bars, left scale) of (a) phenol and 4-methylphenol (4MP) toward triplet-induced oxidation of various target compounds (anilines and sulfonamides), and (b) various AOs toward triplet-induced oxidation of aniline (ANI). Error bars indicate 95% confidence intervals. Symbols represent pseudo-first-order AO depletion rate constants in the presence of the photosensitizer 2AN only (squares, in (a) for clarity of presentation only shown in the first column) and in presence of both 2AN and the respective target compound (triangles).

The depletion of all selected TCs was inhibited in the presence of 10 μ M phenol or 4-methylphenol (Figure 2a), but not in the presence of the negative control compounds, anisole and 4-methylanisole. A significant trend for IE is observed for anilines, being highest for 4CA and decreasing from ANI over NMA to DMA. As discussed above, a probable oxidation intermediate of an aniline is the corresponding aniline radical cation, which can react with a phenolic AO to give the corresponding phenoxyl radical under reformation of the aniline. For a given AO, IE is

expected to increase with the lifetime of the aniline radical cation and with its reduction potential. While such lifetimes are not known, the trend found for IE corresponds adequately well to that of measured standard one-electron reduction potentials (in aqueous solution) for the radical cations of the tested anilines: 4CA: 1.02 V;³⁷ ANI: 1.02 V;³⁷ NMA: 0.95 V;⁴¹ DMA 0.87 V.⁴² For sulfonamides a comparison between IE and the reduction potentials is not possible, because the latter are not available. Moreover, the nature of the radical formed upon sulfonamide oxidation is not known. It could be an aniline type radical as well as a radical resulting from the oxidation of the heterocyclic substituent on the sulfonamide group. This is evident when considering the results for the sulfonamide metabolites (see Table 1 (b), R₂=acetyl). While ³2AN* apparently cannot oxidize the acetylated aniline group of ASD, for which no depletion is observed, it leads to a depletion of ASTZ with a rate constant that is only 25% lower than that of STZ (SI Figures S09, S10 and Table S3). We conclude that the thiazole group in ASTZ is the moiety undergoing oxidation by ³2AN*.

There is no clear answer to the question whether 4MP is a better oxidation inhibitor than phenol. For half of the tested TCs, 4MP shows a lower IE than phenol (Figure 2a). Generally 4MP should be a more effective AO than phenol because the additional *p*-methyl group makes it a better electron donor, which is expressed in a lower standard one-electron reduction potential and oxygen–hydrogen bond dissociation energy.^{43, 44} However, in aqueous solution the acid–base speciation of the phenolic AO and of the radical formed during oxidation of the TC are expected to play an important role in the determination of IE, and, in the absence of detailed information on the reaction kinetics of the various species involved, it is impossible to make reasonable predictions about IE of the different AOs.

Figure 2b shows that all 13 different phenolic compounds are good inhibitors of the triplet induced oxidation of aniline, IE (bars) ranging from 0.4 (2,4DHBA) to 0.85 (3,4DHBA). As in

the case of phenol as an AO, the $^3\text{2AN}^*$ -induced depletion of all tested AOs is catalyzed by aniline. The corresponding depletion rates are weakly correlated to IE ($r^2 = 0.30$). One should be aware that, in contrast to phenol and 4MP, many of the used phenolic AOs reacted at significant rates with $^3\text{2AN}^*$. Substantial formation of reactive AO intermediates, particularly phenoxyl radicals, and AO transformation products may hinder a detailed understanding of the kinetic results. A further unknown in the studied oxidation reactions concerns the role of the superoxide anion radical, which should be generated by reaction of molecular oxygen with the ketyl radical of 2AN.^{1, 32, 35} Superoxide can either add to the radicals resulting from oxidation, leading to oxygenated products, or donate an electron to such radicals leading to their reduction. These reactions may be in mutual competition, as observed for various phenoxyl radicals.⁴⁵ Moreover, the depletion of some of the investigated AOs, such as resorcinol, 2,4DHBA, and 3,5DHBA (all of which have two hydroxyl groups in *m*-position to each other) appears to be autocatalyzed, showing that transformation products of these phenolic AOs can lead to complex kinetics.

An additional series of experiments was performed using TCs that were expected or previously shown⁹ not to undergo inhibition of oxidation in the presence of DOM. The choice was limited to TCs that could be readily oxidized by $^3\text{2AN}^*$, and so the electron-rich phenols TMP, Trolox and 3,4DMOP^{32, 35} were selected. For all these electron-rich TCs, no inhibition of $^3\text{2AN}^*$ -induced oxidation was observed using phenol, 4MP and 3HBA as AOs (see SI, Figure S22), which confirms the expectations. We ascribe the absence of inhibition effect for the oxidative transformation of these electron-rich phenols to the low reduction potential of their phenoxyl radicals (<0.50 V vs. NHE)³⁵, preventing their reduction by the used AOs.

Dependence of the inhibition effect on antioxidant concentration. As in the case of inhibition by DOM^{9, 10}, and also following the reaction mechanism proposed above (see eq. 3), an increase of inhibition with AO concentration was expected. To verify this hypothesis, the pseudo-

first-order rate constants ($k_{Sens,AO}([AO])$) for the $^3\text{2AN}^*$ -induced oxidation of four selected TCs, namely aniline, NMA, DMA and SD, was measured at different concentrations of added phenol and 4MP (Figure 3). For both phenolic AOs the rate constants follow qualitatively the expected decrease with increasing $[AO]$ up to $[AO] \cong 50 \mu\text{M}$. At higher $[AO]$, the decrease continues in the case of phenol, whereas a slight increase is observed for 4MP. A possible explanation of this unanticipated behavior is that 4MP reacts with $^3\text{2AN}^*$ at higher rates than phenol, contributing to an increased formation of 4MP-derived phenoxyl radicals and transformation products which might increase the depletion of the TC. For a quantitative analysis, the previously developed relationships (eq. 7 combined with eq. 8 from our previous study¹⁰) were adapted by substitution of $[\text{DOM}]$ with $[AO]$ to give eq. 9, which was used for data fitting (the nonlinear curve fit procedure for rational functions as provided by the Origin software version 8.0 (OriginLab) was employed).

$$\frac{k_{Sens,AO}([AO])}{k_{Sens}} = \frac{f}{1 + [AO]/[AO]_{1/2}} + (1 - f) \quad (9)$$

The underlying kinetic model considers two parallel reaction channels for the oxidation of a given TC, one of which (channel 1) undergoes inhibition by AO, while the other channel is not affected by AO. In eq. 9, k_{Sens} is the measured rate constant in the absence of AO, while the fitting parameters f and $[AO]_{1/2}$ represent the yield of the channel 1 reaction in the absence of AO and the concentration of AO needed to halve this yield, respectively.

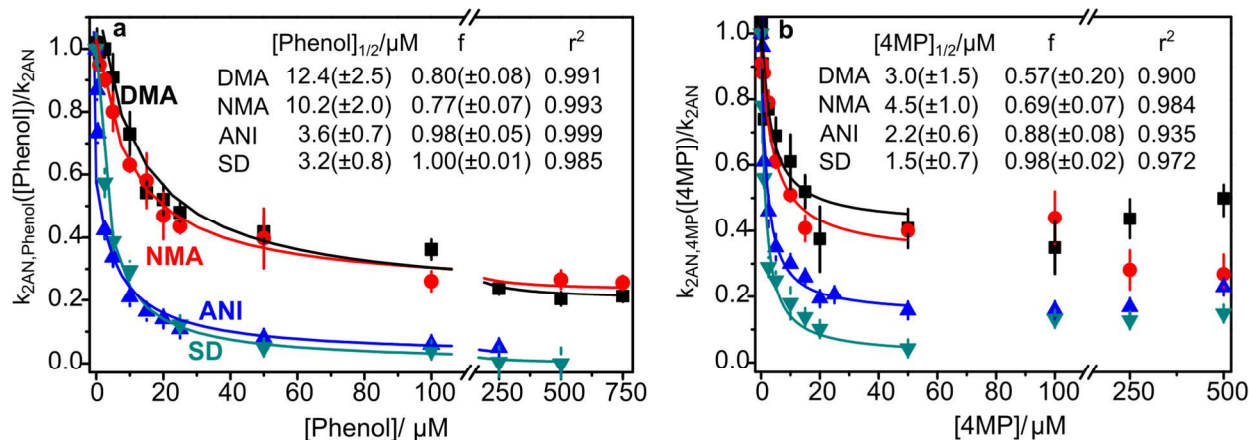


Figure 3. Relative reaction rate constants for the oxidation of the four target compounds *N,N*-dimethylaniline (DMA; ■), *N*-methylaniline (NMA; ●), aniline (ANI; ▲), and sulfadiazine (SD; ▼) as a function of (a) phenol and (b) 4MP concentration. Curves represent nonlinear fits to equation 9. Inserted tables give numeric results of data fitting. Error bars display 95% confidence intervals.

For the reasons given above, fitting of the 4MP data was restricted to the concentration range of 0 – 50 μM. Overall, eq. 9 fits well to the data, with correlation coefficients that are higher for phenol than for 4MP. The fitted values of [AO]_{1/2} are 2 – 4 times higher for phenol than for 4MP, which is reflected in the lower steepness of curves for phenol at low [AO]. This means that, according to the kinetic model, 4MP is the better antioxidant than phenol. The fitted *f*-values for 4MP are lower than for phenol, meaning that the yield of the non-inhibited reaction pathway (1 – *f*) is higher. As discussed in the qualitative analysis of the data, this effect might be related to additional reactions following oxidation of 4MP by ³2AN*. The consistent picture obtained for all 4 TCs, indicating that 4MP is a better inhibitor of ³2AN*-induced oxidation than phenol, are apparently in contrast to the mixed results of the IE values from Figure 2a. However, one should

consider that the IE is affected by f , and so the information given by IE is less detailed than the one obtained from the [AO] concentration dependence.

The fitted [AO]_{1/2} values increase in the order SD < aniline < NMA < DMA for phenol, while the order of NMA and DMA is reversed for 4MP. For the anilines and phenol as the AO, this order is in line with the decrease in reduction potential of the corresponding aniline radical cations (see above), providing further evidence that these could be the relevant oxidation intermediates involved in the inhibition of oxidation. Considering SD as a substituted aniline, one can roughly predict the reduction potential of its radical cation to be on the order of 1.2 V vs. NHE,¹ perfectly matching the order predicted from [AO]_{1/2}.

Phototransformation of target compounds induced by DOM: Inhibition by phenolic antioxidants. This sub-section deals with the application of combination mode 2 (see Scheme 1 and the *Introduction*) to obtain information about a possible inhibition effect caused by antioxidants on ³DOM*-induced oxidation of TCs. The implementation of the irradiation experiments is more critical than for the case of a model photosensitizer, since the concentration of DOM has to be kept quite low to avoid excessive inhibition of the reaction by DOM itself, which acts of course both as a photosensitizer and an inhibitor. Owing to the less efficient photosensitizing activity of DOM with respect to model aromatic ketones,³² required irradiation times to study the depletion kinetics of the TC were found to be at least one order of magnitude higher than for the corresponding experiments using 2AN. Under these conditions, the phototransformation of some TCs, such as aniline and SCPD, in blank solutions (i.e. without DOM) was very important. Consequently, such TCs were considered to be inappropriate for the sake of the present study. Also, under DOM photosensitization and such long reaction times, 4MP was depleted rapidly in comparison to the TCs, and was therefore considered to be inappropriate as a model AO in this case. SMX and SD were found to undergo negligible

transformation in irradiated blank solutions and were therefore selected. The detailed kinetic data are given in SI, Figures S23-S25, while Figure 4 displays the inhibition, expressed as IE, for the depletion of these two TCs obtained using phenol (10 μM) as the AO and various DOM solutions and natural waters as the photosensitizers.

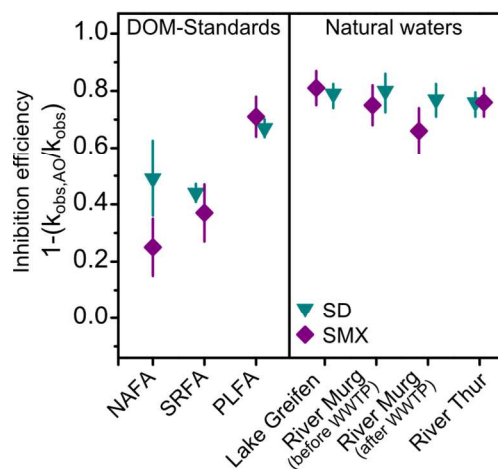


Figure 4. Inhibition efficiency of 10 μM phenol on the oxidation of sulfadiazine (SD) and sulfamethoxazole (SMX) irradiated in standard DOM solutions (5 mg C L^{-1}) and natural waters (1.9 – 3.8 mg C L^{-1}). Error bars display 95% confidence intervals.

The inhibition follows for both TCs the same trend, with IE being significantly lower for the mainly allochthonous fulvic acids than for the mainly autochthonous PLFA and the natural waters. The interpretation appears straightforward, because the allochthonous DOMs are expected to inhibit more efficiently the triplet-induced oxidation than less aromatic DOMs¹⁰ such as PLFA and the organic matter in the used freshwaters, thus partially neutralizing the potential for further inhibition. For PLFA and the natural waters, IE for SD is almost the same as determined with 2AN as a photosensitizer (Figure 2a), suggesting a low concentration of

inhibitors (and AOs) in such systems. In contrast to the results obtained using 2AN as the photosensitizer, the depletion of the AO (phenol in this case, see Figure S25 of SI) was already important in the presence of DOM only, and was not significantly accelerated by the presence of TC.

Environmental relevance. Our results clearly show that phenolic compounds with antioxidant character are capable of inhibiting the excited triplet-induced transformation of several anilines and sulfonamide antibiotics in aerated aqueous solution. Thereby excited triplet state quenching was excluded as a possible cause of inhibition. The effect was observed using a model photosensitizer, the aromatic ketone 2AN, as well as various types of DOM (in dissolved extracts or as present in freshwater samples) as natural photosensitizers. The used phenolic AOs are apparently capable of mimicking DOM as an inhibitor of excited triplet-induced oxidations.⁹
¹⁰ This property of phenolic AOs can be exploited as a diagnostic tool to characterize the degradation pathway of anilines, sulfonamides and possibly other important contaminants in the aquatic environment. For instance, if the indirect phototransformation of a given contaminant is efficiently inhibited by addition of phenol (or another appropriate AO) at a relatively low concentration (<10 μ M), this finding would support excited-triplet induced oxidation as a relevant transformation mechanism. Within our research group, this method is currently under development and is being applied for an improved understanding of the aquatic photochemistry of sulfonamides, including those which have been the subject of recent studies.^{22,23}

We now address the question whether phenolic AOs are sufficient to explain the inhibition of oxidation that occurs in the presence of DOM. Owing to the complexity of DOM, not only phenolic AO moieties but also a variety of further mechanisms might be responsible for its inhibitive action. The phenolic content of various DOM extracts has been estimated by acid/base

titration.¹⁵ For NAFA and SRFA it was found to be $\approx 3 \text{ meq (g C)}^{-1}$, implying that 1 mg C corresponds to 3 μmol of phenolic moieties in the DOM. Only a certain part of these moieties is expected to have AO character, thus the value has to be considered as an upper limit for the phenolic AO content. Using the $[\text{DOM}]_{1/2}$ values previously determined¹⁰ and converting them to phenolic moiety concentrations, one obtains values of 0.4 – 0.6 μM and $\approx 6.5 \mu\text{M}$ for DMA and SMX as TC, respectively (data for 2AN as a photosensitizer). In the case of DMA, the level of DOM phenolic moieties to achieve a 50% inhibition is much lower (by a factor of 5 – 30) than $[\text{AO}]_{1/2}$ determined here (Figure 3) with phenol and 4MP. A possible explanation of such an underestimation of the antioxidant activity of DOM is that the used model AO are less reactive with oxidation intermediates of DMA than the corresponding moieties in DOM. This may well be the case, since NAFA and SRFA possess $\approx 1 \text{ meq (g C)}^{-1}$ of electron donating groups that can be oxidized at potentials as low as 0.61 V at pH 8,¹² which is much lower than the potential required to oxidize phenol and 4MP, and also much lower than the redox potential of the DMA radical cation. An analogous comparison between $[\text{DOM}]_{1/2}$ for SMX and $[\text{AO}]_{1/2}$ for SD shows that they have similar values. Thus, the assumption that AO moieties of DOM are responsible for its inhibition of oxidation appears to be reasonable.

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Supporting Information Available

3 texts, 3 tables and 25 figures are available for further information addressing materials, experimental procedures, and additional data. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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